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Research Paper

IN VIVO EVALUATION OF ANTIDIARRHOEL ACTIVITY OF THE *MANGIFERA INDICA* SEED KERNEL

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Abstract

The aim of this study was to investigate the antidiarrhoeal potentials of ethanolic extract of *Mangifera indica* seed kernel. The ethanolic extract of *Mangifera indica* seed kernel [100, 200, and 400 mg/kg body weight] was administered orally to three groups of rats [five animals per group] in order to evaluate the activity of the extract against castor oil-induced diarrhea. Two other groups received normal saline [5mg/kg] and Loperamide [5mg/kg] as positive control. One other group served as a disease control. The effect of the extract on castor oil-induced diarrhoea, gastrointestinal transit and intestinal fluid accumulation was assessed respectively. In this study, at oral doses of 100, 200, and 400 mg/kg body weight, the plant extract showed pronounced significant [$p<0.05$] antidiarrhoeal activity compared to the control group. The results showed that the ethanolic extract of *Mangifera indica* seed kernel has a significant antidiarrhoeal activity which supports its use in traditional herbal medicine practice.

Key words: Antidiarrhoeal activity, Castor oil, Intestinal transit, *Mangifera indica* seed kernal.

INTRODUCTION

Diarrhoea is a symptom of frequent passage of semisolid or liquid feacal material through the gastrointestinal tract and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and a decrease in the absorption of fluid and thus a loss of electrolytes particularly Na^+ and water [1] . Diarrhoea is also called as loose motions. It is one of the clinical symptoms of gastrointestinal tract infection, but also can reflect primary disorders outside the digestive system. According the WHO reported diarrhoea is one of the leading causes of mortality and morbidity in developing countries especially in children under five years, which kill around 1.8 million people globally each year [2]. The major causative agents of diarrhoea in human include *Shigella flexneri, Staphylococcus aureus, Eschrichuia coli and Salmonella typhi* [3]. Many synthetic chemicals like such as codeine, loperamide, diphenoxylate, bismuth subsalicylate, reacedotril are available for the treatment of diarrhoea but these antibiotics have been threatened by the emergence of microbial resistance to existing chemotherapeutic agents because of their indiscriminate and

inappropriate use. The use of these antibiotics is linked to side effects, including allergy, immune suppression, hypersensitivity, nausea, dry mouth abdominal discomfort and headache. A vast majority of the people in developing countries rely on herbal drugs for the management of diarrhoea. Considering this fact, the World Health Organization has constituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches [4]. Herbal medicines are used for throughout the world in the past decade as an alternative approach of peoples to improve the good health and quality of life. Herbs have been containing the original source of drugs. Extensive studies of the adverse effects of these herbal medicines and establishment of a reliable correlation between biomarkers and plants are essential for ensuring the efficiency and quality of herbal medicines. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. The acceptance of traditional medicine as an alternative form of health care, researchers to investigate the antidiarrhoeal activity of herbal extracts. Plants containing flavonoids, terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity [5]

Mangifera indica Linn. belongs to the family Anacardiaceae, the fruit of which is consumed throughout the world. It has been cultivated extensively in India, Myanmar and some parts of America, Africa, Pakistan and Bangladesh [6]. It is commonly known as aam (Hindi), Manga (Tamil) [7], Ampleam (Spanish) [8].It is a long-lived evergreen tree that can reach up to a height of 50 to 100 feet. Seed kernel of this plant contributed about 17 - 22% of the fruit. The seed kernel is not yet used for any commercial purposes [9]; it is discarded as a waste and contributes a source of pollution. This waste should be considered as a specialized residue due to their high level of phenolic compounds and stable fat rich in saturated fatty acids [10]. Traditionally *M. indica* Seed kernel (MISK) is used to cure chronic diarrhoea, to expel tapeworms and other worms in ulcers [11]. Powdered seed kernel is used as antihelminthic. It also possesses antioxidant [12] and antidiarrhoel activity [13]. It has been used for its antidiarrhoel activity in Indian traditional medicine [14]. It is noticed that, traditionally tribal people of India ate mango seed kernel in roasted form during starvation, as it is rich in Starch [15]. The kernel powder is invoked as astringent in bleeding piles [16]. MISK is presented in traditional medicine as a cure for vomiting, dysentery and burning [17]. In villages MISK is used along with honey to treat helminthic infections [18]. In the present study, MISK is analysing the for its antidiarrhoeal effects on castor oil-induced diarrhoeal activity in rat.

MATERIALS AND METHODS

Plant material collection

The fruits of *M. indica* were purchased from the local market of Tiruchirappalli, Tamilnadu, India and seeds were separated from the fruit. The hard seed coat was removed and the seeds were dried. These dried seeds were coarsely powdered and stored in a closed container for further use.

Extraction

200gm of *M. indica* seed kernel powder of soaked in 1200ml of Ethanol and incubated for 72 hrs. Then it was filtered and evaporated to dryness. The extracts obtained were subject to pre-clinical screening. This extract is called *Mangifera indica* seed kernel ethanolic extract (MISKEE)

Animals

Albino rats of both sexes weighing 100-200g were utilized for the study. They were purchased from the TANUVAS, Chennai. They were stored in well equipped polypropylene cages at room temperature of 24°C and exposed to both light and also dark cycle. All the animals were allowed to acclimatise for two weeks in animal house of Srimad Andavan Arts and Science College, Tiruchirappalli. The animals were fed with a standard pelleted diet and water ad libitum. The container for the food and water was washed daily, as the food and water are renewed every day, to ensure hygiene and maximum comfort for the animal. The study protocol was approved by CPCSEA NO 790/03/ac/CPCSEA.

Anti diarrhoeal activity

Castor oil-induced diarrhea

Albino rats of either sex (200-250g) were subdivided into seven groups of six animals each. They were fasted for 24h prior to the test, but allowed free access to water. Group I received normal saline, which serves as control; Group II received 2ml of castor oil, which serve as disease control; Group II received standard drug of Loperamide 3mg/kg. Test groups IV and V received aqueous extract and test groups VI and VII received methanol extracts oral doses of 100 and 200 mg/kg b.w. All doses were administered orally. The animals were then housed singly in cages lined with transparent paper. One hour after pre-treatment with the extract, the animals were challenged with 1 ml of castor oil orally. Thereafter, they were observed for 4h for the presence of diarrhea defined as watery (wet), unformed stool [19]. The frequency of defecation and number of diarrheal faeces excreted in the recorded time were scored and compared with control group. The results were expressed in percentage of inhibition.

Castor oil induced enteropooling

The Albino rats were divided in to five groups and each groups comprise of six rats only. They were fasted overnight and allowed the only access of water. Group I received normal saline, which served as control; Group II received 2ml of castor oil, which serve as disease control; Group II received standard drug of Loperamide 3mg/kg. Test groups IV and V received aqueous extract and test groups VI and VII received methanol extracts oral doses of 100 and 200 mg/kg b.w. Castor oil was administered orally after 30 min of drug administration. Two hours later rats were sacrificed, and the small intestine was removed after tying the ends with thread and weighed. The duodenal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestine was calculated [20, 21]

Study of Gastrointestinal Tract Mobility

Using charcoal meal as a diet marker. Albino rats of either sex (100-250g) were randomly subdivided into six groups of six rats each. They were fasted for 24 hours prior to the test, but were allowed free access to water. Group I received normal saline, which serves as control; Group II received 2ml of castor oil, which serves as disease control; Group II received standard drug of Loperamide 3mg/kg. Test groups IV and V received aqueous extract and test groups VI and VII received methanol extracts oral doses of 100 and 200 mg/kg b.w. Thirty minutes after drug administration, 1ml of charcoal meal (10% activated charcoal in 5% aqueous gum arabica was administered to all the animals in the study and thirty minutes later, all the rats were sacrificed and the small intestine was dissected out and the distance covered by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as a percentage of the distance traveled [22].

Statistical Analysis

All the results were expressed as mean ±S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA] and P values <0.05 were considered significant.

RESULTS

The ethanolic extract of *M. indica* seed kernel was found to be effective against castor oil induced diarrhoea on experimental rat at various doses of 100, 200 & 400 mg/kg body. At the dose of 400 mg/kg body weight, the ethanolic extract of MISK produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation and consistency of faeces in albino rats. At the same dose, the extract showed significant antidiarrhoeal activity [P □ 0.05] showing 43.65% reduction in diarrhoea comparable to that of the standard drug loperamide that showed 26.33 % reduction in diarrhoea [Table 1].

Results of the present study revealed that the ethanolic extract of *M. indica* seed kernel extracts significantly reduced the gastrointestinal transit of charcoal in rat [Table 2]. This activity was significant [P □ 0.05] showing 50.95% of reduction in gastrointestinal transit comparable to the standard drug loperamide showed 27.51%.

The ethanolic extract of *M.indica* significantly [P□0.05] inhibited the castor oil induced enteropooling (Table 3) in rat at 400mg/kg. Table 3 shows the intestinal fluid in diseased

animals was 1.59±0.07ml. The inhibition of intestinal accumulation was 0.69±0.21ml in the dose of 400 mg/kg of the ethanolic extract, respectively. The standard drug of loperamide was also significantly inhibited intestinal fluid accumulation 1.07±0.036ml.

Table 1: Effect of ethanolic extract of *M. indica* seed kernel at different doses level on Castor oil induced diarrhoea in rat

S.NO	Groups	Treatment	Mean defecation	% inhibition of defecation
1	I	Normal control	3.10±0.36	**---**
2	II	Disease control	3.60±0.49	**--**
3	III	Standard drug (Loperamide)	4.23±0.039	**26.33±1.633**
4	IV	MISKEE(100 mg/kg body weight]	3.83±0.069*	**34.66±2.503**
5	V	MISKEE (200 mg/kg body weight)	3.25±0.043*	**42.17±1.722**
6	**VI**	**MISKEE (400 mg/kg body weight)**	**3.15± 0.80***	**43.65±2.161**

Values are expressed as mean ± SEM [n=6] * P<0.05 statistically significant when compared Group IV, V & VI with Group III

Table 2: Effect of ethanolic extract of *M. indica* seed kernel at different doses level on Charcoal induced gut transit changes in rat

S.NO	Groups	Treatment	Length of intestine	Distance travelled by charcoal	% intestinal transmit
1	I	Normal control	----	------	-----
2	II	Disease control	87.2±1.56	76.4±2.4	----
3	III	Standard drug (Loperamide)	84.2±0.05	52.5±0.3	27.5±0.2
4	IV	MISKEE(100 mg/kg body weight)	85.7±0.36	45.4±0.3*	38.5±0.2
5	V	MISKEE (200 mg/kg body weight)	85.8±0.53	38.6±0.2*	50.3±0.3
6	VI	MISKEE (400 mg/kg body weight)	85.1±1.43	37.5±3.1*	50.9±0.5

Values are expressed as mean ± SEM [n=6] * P<0.05 statistically significant when compared Group IV,V & VI with Group III**.**

Table 3: Effect of ethanolic extract of *M. indica* seed kernel at different doses level on Castor oil induced enteropooling in rat

S.NO	Groups	Treatment	Mean weight of intestine [g]	Volume of intestinal content [ml]	% of inhibition
1	I	Normal control	----	-----	-----
2	II	Disease control	5.1±2.16	1.5±0.07	---
3	III	Standard drug (Loperamide)	7.1±0.04	1.1±0.03	36.3±0.05
4	IV	MISKEE(100 mg/kg body weight)	8.2±0.04	0.8±0.02*	46.5±0.06
5	V	MISKEE (200 mg/kg body weight)	8.4±0.03	0.6±0.03*	55.3±0.18
6	VI	MISKEE (400 mg/kg body weight)	8.4±1.9	0.6±0.21*	56.6±0.11

Values are expressed as mean ± SEM [n=6] * P<0.05 statistically significant when compared Group IV, V & VI with Group III.

DISCUSSION

In the traditional medicine system, *Mangifera indica* is used in the management of diarrheoa. The present study to assess the antidiarrhoel activity of the *M.indica* seed kernel. Our results showed that the ethanolic extract of MISK inhibited significantly [p < 0.05] castor oil-induced diarrheoa in rats. Diarrhoea may be characterized as the abnormally frequent defecation of faeces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines. Despite the multiplicity of aetiologies, the four major mechanisms responsible for the pathophysiology in water and electrolytes transport are [i] increased luminal osmolarity [osmotic diarrhoea], [ii] increased electrolytes secretion [secretory diarrhoea], [iii] decreased electrolytes absorption, and [iv] deranged intestinal motility causing a decreased transit time [23]. These include inhibition of intestinal Na^+, K^+ ATPase activity, thus reducing normal fluid absorption [24] , activation of adenylate cyclase or mucosal cAMP-mediated active secretion [25] , stimulation of prostaglandin formation [26], and platelet activating factor [27] . Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil [28] . Castor oil causes diarrhoea due to its active metabolite, ricinolic acid. It leads to changes the electrolyte permeability of intestine and also it stimulates the release of prostaglandin and peristaltic activity of small intestine. In this study, the ethanolic extract of *Mangifera indica* exhibited a significant antidiarrhoeal activity. The results were comparing to that of the standard drug loperamide with regard to the severity of diarrhoea. Furthermore, the ethanolic extract was significantly reduced intestinal transit as evidenced by the decrease in the distance traveled by charcoal meal. These results also show that the MISK extract suppressed the propulsion of charcoal meal thereby increasing the absorption of water and electrolytes and also it increase the weight of the intestine when compare to the standard drug loperamide . Antidiarrhoeal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and/or terpenes [29, 30] . The antidiarrhoeal activity of flavonoids has been described to their ability to inhibit intestinal motility and hydro-electrolytic secretions [31,32] which are altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E 2 [33] . In addition, flavonoids present antioxidant properties

[34] which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism [35]. These constituents may be responsible for the antidiarrhoel activity of the ethanolic extract of *M. indica*.

CONCLUSION

The results of this investigation revealed that ethanolic extract of *M.indica* contains pharmacologically active substances with antidiarrhoeal properties. Further research is needed to fully investigate the mechanisms involved in the pharmacological activities, to isolate and characterize the active constituents of *M.indica*. The isolated compound may serve as useful prototypes of antidiarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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